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The Protein Biochemistry of the Postsynaptic Density in Glutamatergic Synapses Mediates Learning in Neural Networks

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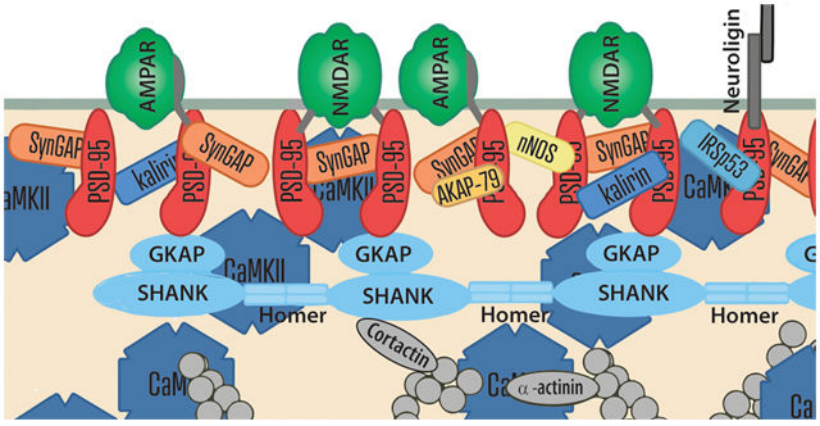
Abstract

The strength of each excitatory synapse in the central nervous system is regulated by its prior activity in a process called synaptic plasticity. The initiation of synaptic plasticity occurs when calcium ions enter the postsynaptic compartment and encounter a subcellular structure called the postsynaptic density (PSD). The PSD is attached to the postsynaptic membrane just underneath the concentrated plaque of neurotransmitter receptors. It is comprised of a core set of 30–60 proteins, approximately 20 of which are scaffold proteins. The rest include protein kinases and phosphatases, some of which respond to calcium ion; small GTPases and their regulators; chaperones; ubiquitins; and proteases. The assembly of the PSD involves competitive binding among a variety of specific protein binding sites to form a dynamic network. A biochemical challenge for the future is to understand how the dynamic regulation of the structure, composition, and activity of the PSD mediates synaptic plasticity and how mutations in PSD proteins lead to mental and neurodegenerative diseases.

Graphical Abstract



Excitatory synapses that use glutamate as a neurotransmitter make up the majority of synapses in the brain. Typical pyramidal neurons have as many as 10000 synapses impinging on their dendrites and somas. When a synapse is active, it causes a small depolarization of

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the postsynaptic neuronal membrane that can add to those caused by all other active synapses on the neuron. When the total depolarization reaches a threshold, the neuron fires an action potential along its axon to activate its own presynaptic terminals. The “strength” of each synapse, that is the size of the depolarization it causes when it is activated, varies depending on the synapse’s history. The pattern of activity at a synapse can increase or decrease its strength by a process called synaptic plasticity.[1-3]

In particular, if a synapse is repeatedly activated presynaptically at the same time as the postsynaptic neuron is strongly depolarized (for example, by a back-propagating action potential), the synapse becomes stronger by a process called long-term potentiation [LTP (Figure 1)]. Conversely, if a synapse is repeatedly activated presynaptically while the postsynaptic neuron is weakly depolarized (~30 mV or less), the synapse becomes weaker by a process called long-term depression (LTD). Surprisingly, although these processes adjust synaptic strength in opposite directions, both are driven by the flux of calcium ion through a specialized glutamate receptor called the NMDA-type glutamate receptor (NMDAR).[4] A synapse is tipped toward LTP when the flux of Ca^{2+} is large and peaks rapidly; it is tipped toward LTD when the flux of Ca^{2+} is relatively small and continues in small amounts over several minutes.[1] The flux of Ca^{2+} through the NMDAR is linked to the pattern of synaptic activity by the rules governing opening of the NMDAR channel.[5,6] The behavior of the NMDAR is now well understood, and the structure of its channel has been determined at the atomic level.[7] However, an important challenge remaining for biochemists interested in studying the brain is to unravel the complex biochemical mechanisms that underlie LTP and LTD and that determine how they are produced by differing levels of synaptic activity.

The set of signaling enzymes that initiates LTP and LTD resides in a structure called the postsynaptic density (PSD) attached to the plaque of receptors located at the synaptic cleft.[8,9] The emerging view of this structure suggests that it constitutes a type of “membraneless compartment”[10,11] in the classical cell biological sense.[12-14] The PSD is comprised of a set of scaffold proteins that concentrate and direct the reactions of a network of regulatory enzymes.[9,15]

Studies of the molecular composition of the PSD were initiated by the work of Philip Seikevitz and Carl Cotman, who used cell fractionation techniques to isolate a subcellular fraction from brain homogenates enriched in structures with the approximate thickness and length of PSDs observed in the electron microscope (e.g., ref 16). After a brief period of controversy with respect to whether the PSD fraction represented a subcellular structure or simply an uninteresting protein aggregate, the issue was settled when my laboratory identified a new scaffold protein, PSD-95, highly enriched in the PSD fraction, and showed that it is localized nearly exclusively at the postsynaptic membranes of excitatory synapses.[17] These findings were “pre-genome” and involved microsequencing of proteins from PSD fractions separated on sodium dodecyl sulfate gels followed by cDNA cloning of the full length coding region of PSD-95. We raised antibodies against the protein and established its subcellular location by immunocytochemistry. Through the next decade, several core proteins that are highly localized in the postsynaptic density of intact tissue were identified in the PSD fraction by many laboratories.[8,9,18,19] The completion of the genome sequencing

projects in ~2003 left us with a reasonably accurate list of the major core PSD proteins. Since then, a major task has been to understand how the machinery comprised of these proteins works to regulate synaptic strength.

Before outlining some of the biochemical opportunities for studying this molecular machinery, I'll address an often-voiced notion that the PSD contains so many individual proteins that it seems an impossibly complex structure to decipher. Early proteomic studies employing mass spectrometry resulted in identification of one to two hundred proteins said to be associated with postsynaptic machinery.[20,21] However, these conclusions should to be tempered by two considerations. First, like any fractionation technique, cell biological methods for isolating postsynaptic proteins, or postsynaptic density proteins, are capable of enriching only stable structures that are believed to represent the subcellular organelle of interest. They never result in completely pure preparations. On the other hand, the detection of proteins by proteomic mass spectrometry is extremely sensitive and so will identify minor and/or contaminating components as well as major constituents of any subcellular fraction. The early proteomic studies of synapses did not distinguish between highly abundant, enriched proteins in the fraction and more minor proteins. In addition, the high number of proteins includes individual members of families of closely related proteins that contain several homologues. More refined studies, which take advantage of quantitative methods, [22,23] and consideration of immunocytochemical studies to cross-check the subcellular localizations of proteins, point to a smaller set of proteins that constitute the "core" PSD proteins. Investigators differ on the exact size of this set, but it appears to me to be no smaller than ~30 and no larger than ~60. This set of proteins is likely present in nearly all PSD structures in excitatory postsynaptic spines, some at high abundance and others at just a few copies.

Four classes of scaffold proteins comprise most of the central scaffold that holds together the postsynaptic density; MAGUKs, SHANKs, SAPAPs, and Homers (Figure 2). The MAGUK (membrane-associated guanylate kinase) family includes PSD-95 (SAP90), PSD-93 (Chapsyn), SAP97, and SAP102.[24,25] Each contains three PDZ domains that are the business end of the MAGUK scaffolds because they bind to receptors and to a variety of signaling enzymes that regulate synaptic strength. Each PDZ domain binds a five- to seven-residue motif usually occurring at the C-terminus of ligand proteins. The guanylate kinase domains (which are inactive) bind directly to the SAPAPs (also called GKAPs), which in turn link via their C-termini to the PDZ domain in SHANK.[26] The affinity of SAPAPs for the PDZ domain is strengthened by additional binding between residues adjacent to the PDZ ligand and the PDZ domain in SHANK.[27] The SHANKs reside ~12 nm more distal to the membrane than the MAGUKs.[28] They can adopt sheetlike tertiary structures mediated by association of their SAM domains, suggesting that they form an underlying "base" to which the more proximal scaffold proteins are anchored.[29,30] The Homers multimerize to form rod shapes bearing protein binding EVH domains at either end.[31,32] They link metabotropic glutamate receptors, IP3 receptors, and TRP channels to the SHANK scaffold.[33]

The emerging picture of how these core proteins are arranged is still poorly resolved (Figure 3). Thus, an important challenge for biochemists is to understand at the structural level how the core proteins interact specifically with each other and how dynamic changes in the

organization of the PSD occur as synaptic strength is altered in response to activity. These issues represent a fertile opportunity for biochemists willing to tackle the complexity of excitatory synaptic machinery in the central nervous system. The conceptual and methodological challenges are similar to those posed by many other dynamic cytosolic protein machines, including chromosomes, spindles, kinetochores, ribosomes, and splicesomes. How do the individual scaffold proteins interact with each other, and how is their interaction regulated? How does competition for binding to certain protein binding domains shape the ultimate configuration of the machinery?[34] What role do liquid–liquid phase transitions[11] play in formation of the "membraneless" cellular compartment? Finding solutions to these questions takes on additional salience because mutations in several of the proteins most central to the PSD can cause or contribute to Alzheimer's disease,[35] mental illnesses,[36] autism spectrum disorders,[37] and intellectual disability.[38]

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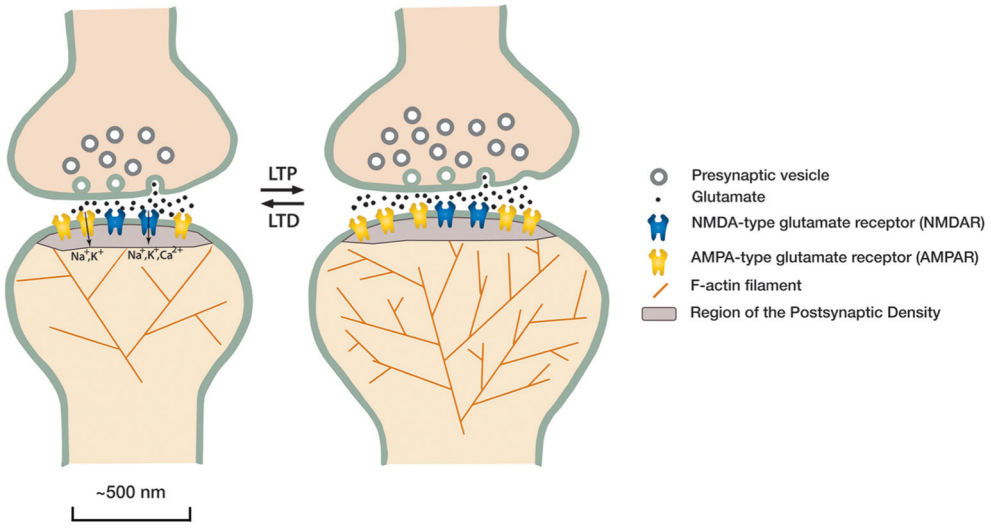

Figure 1.

Synaptic plasticity includes the processes of LTP and LTD. LTP involves the addition of new synaptic AMPA-type glutamate receptors (AMPARs), which causes greater depolarization upon activation of the synapses. It also includes an increase in the size of the head of the postsynaptic spine, supported by an increase in the size and branching of the actin cytoskeleton. Long-term depression (LTD) is a long-lasting decrease in synaptic strength that involves a decrease in the number of synaptic AMPARs and shrinkage of the spine head. The area of the PSD contains scaffold proteins and signaling molecules that organize and catalyze these changes. Adapted from Figure 1 of ref 3. Copyright Cold Spring Harbor Laboratory Press 2016.

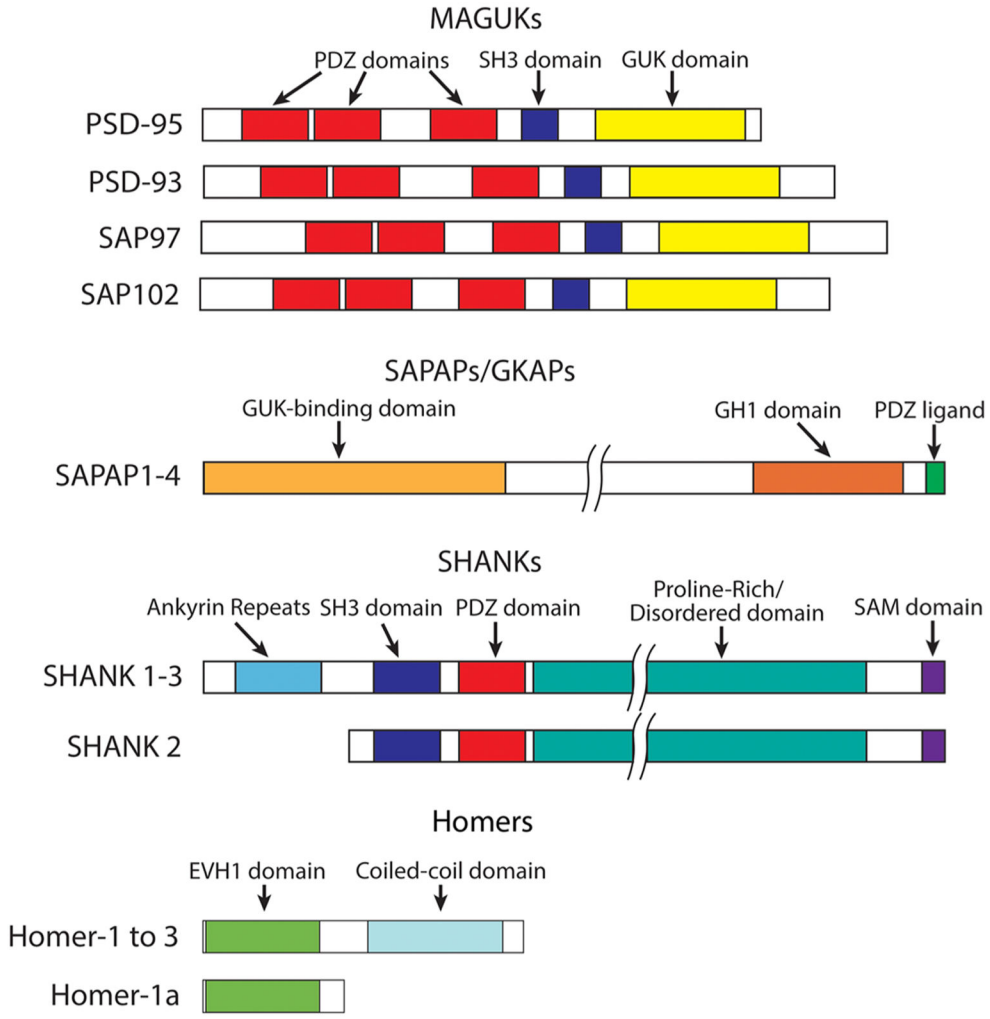

Figure 2.

The PSD includes four major classes of scaffold proteins that are found only, or primarily, in the PSD. MAGUKs (membrane-associated guanylate kinases) contain three PDZ (PSD-95, discs large, ZO1) domains, an SH3 (Src homology 3) domain, and a GUK (guanylate kinase-like) domain. SAPAPs (synapse-associated protein-associated proteins) or GKAPs (guanylate kinase-associated proteins) contain a domain of five 14-residue repeats that bind directly to the GUK domain of PSD-95, a GH1 (region of homology to a *Caenorhabditis elegans* protein) domain, and a seven-residue PDZ ligand at the C-terminus that associates with the PDZ domain of SHANKs. SHANKs (SH3 domain, ankyrin repeats) contain multiple ankyrin repeats at the N-terminus, an SH3 domain, a PDZ domain that binds to SAPAPs, an extended proline-rich disordered domain, and an N-terminal SAM (sterile alpha motif) that can self-associate. Homers contain an EVH1 domain that binds metabotropic and IP3 receptors and a coiled-coil domain by which the Homers multimerize. Only Homer-1a lacks the coiled-coil domain.

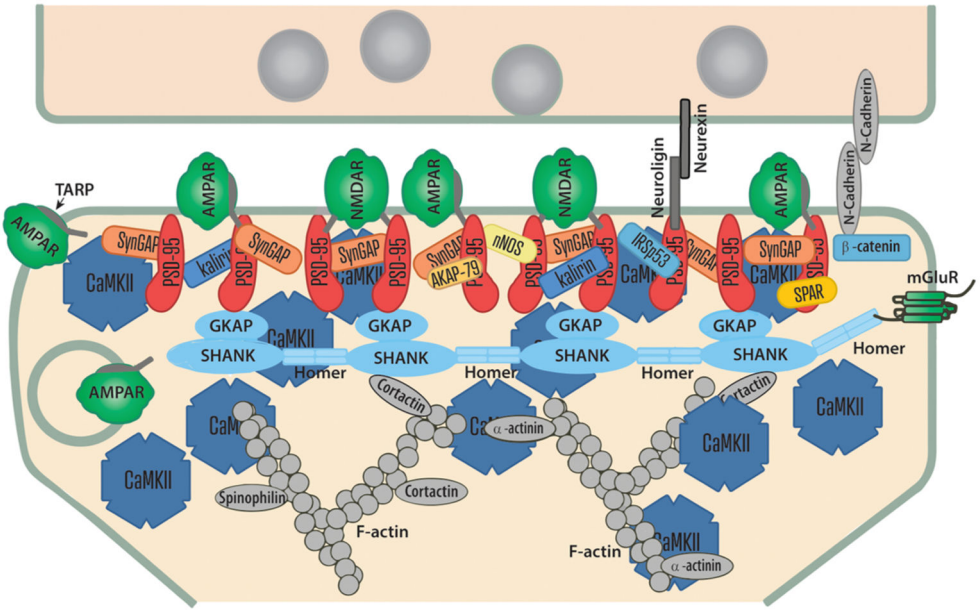

Figure 3.

Cartoon of the organization of the postsynaptic density (PSD). Signaling proteins are immobilized near receptors at the synapse by a core group of scaffold proteins, including PSD-95, GKAP, SHANK, and Homer. Electron microscopic immunocytochemistry has revealed an approximate organization of the scaffold proteins (see the text). However, the precise organization of individual proteins and the mechanisms by which the dynamics of the structure are regulated remain to be determined. Adapted from Figure 4 of ref 3.